

Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

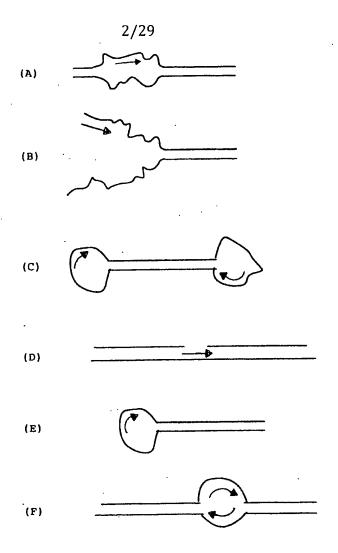
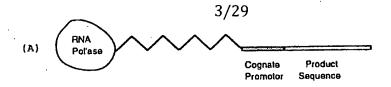


Figure 2 (A-F)

Functional Forms of the Construct



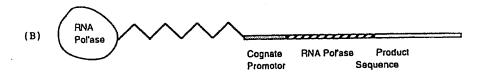




Figure 3 (A-C)

Three Constructs with an RNA Polymerase Covalently Attached to a Transcribing Cassette

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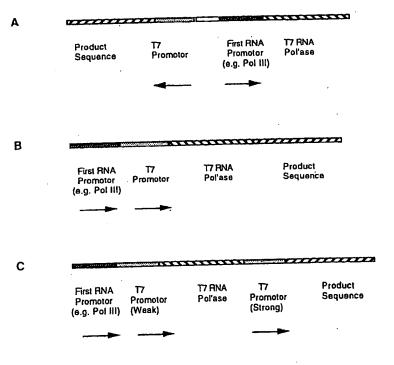


Figure 4 (A-C)

Three Constructs with Promoters for Endogenous RNA Polymerase

M13mp	18. Seq Len	gth: 7250			
1.	AATGCTACTA	CTATTAGTAG .	AATTGATGCC	ACCTITICAG	CTOCCOCC
51.	AAATGAAAAT	ATAGCTAAAC	AGGITATIGA	CCATTTGCGA	AATGTATCTA
101.	ATGGTCAAAC	TAAATCTACT	OGTTOGCAGA	ATTOCCAATC	AACTGTTACA
151.	TGGAATGAAA	CTTOCAGACA	COGTACTTTA	GTTGCATATT	TAAAACATGT
201	TGAGCTACAG	CACCAGATTC	AGCAATTAAG	CTCTAAGCCA	TOOGCAAAAA
251	TGACCTCTTA	TCAAAAGGAG	CAATTAAAGG	TACTCTCTAA	TOCTGACCTG
301.	TTGGAGTTTG	CTICCCGCTCT	egnoecht	GAAGCTOGAA	TTAAAACGCG
351.	ATATTTGAAG	тсптососс	ттостсттаа.	TCTTTTTGAT	GCAATCCGCT
401.	TIGCTICTGA	CTATAATAGT	CAGGGTAAAG	ACCTGATTTT	TGATTTATGG
451.	TCATTCTCGT	TTTCTGAACT	GTTTAAAGCA	TTTGAGGGGG	ATTCAATGAA
501.	TATTTATGAC	GATTCCGCAG	TATTGGACGC	TATOCAGTCT	AAACATITTA
551.	CTATTACCCC	CTCTGGCAAA	ACTTCTTTTG	CAAAAGOCTC	TOGCTATTIT
601.	GGTTTTTATC	GICGICIGGI	AMACGAGGGT	TATGATAGTG	TIGCTCTTAC
651.	TATECCTOGT	AATTCCTTTT	COCCITATET	ATCTGCATTA	GITGAATGTG
701.	GTATTCCTAA	ATCTCAACTG	ATGAATCTTT	CTACCTGTAA	TAATGTTGTT
751.	COGITAGITC	GTTTTATTAA	CGTAGATTIT	TCTTOCCAAC	GTOCTGACTG
801.	GTATAATGAG	CCAGTTCTTA	AAATOGCATA	AGGTAATTCA	CAATGATTAA
851.	AGTTGAAATT	AAACCATCTC	AAGCCCAATT	TACTACTOGT	TCTCGTGTTC
901.	TOGTCAGGGC	AAGCTTATT	CACTGAATGA	GCAGCITTGT	TACGITGATT
951.	TGGGTAATGA	ATATCCGGTT	CTTGTCGAAG	ATTACTCTTG	ATGAAGGTCA
1001	GOCAGOCTAT	ecectiestic	TGTACACOGT	TCATCTGTCC	TCTTTCAAAG
1051	TTGGTCAGTT	COGTICCCTT	ATGATTGACC	GICIGOGOCT	OGTIOOGGCT
1101	AAGTAACATG	GAGCAGGTOG	OGGATTTOGA	CACAATTTAT	CAGGOGATGA
1151	TACAAATCTC	CGTTGTACCTT	TGTTTCGCGC	TTGGTATAAT	COCTOCOCCT
1201	CAAAGATGAG	TGTTTTAGTG	TATTCTTTCG	CCICTITCGT	TITAGGTTGG

Figure 5

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1251	TGCCTTCGTA	GTGGCATTAC	GTATTTTACC	CGTTTAATCG	AAACTTCCTC
1301	ATGAAAAAGT	CTTTAGTCCT	CAMAGOCTICT	GTAGCOGTTG	CTACCCTCGT
1351	TOOGATGCTG	TCTTTCGCTG	CTGAGGGTGA	OGATOCOGCA	AMAGOOGOCT
1401	TTAACTCCCT	GCAAGOCTCA	COCACOGAAT	ATATOGGTTA	TEOGTEGGOG
1451	ATGGTTGTTG	TCATTGTOGG	OGCAACTATC	GETATCAAGC	TGTTTAAGAA
1501	ATTCACCTOG	AAAGCAAGCT	GATAAACCGA	TACAATTAAA	EGCTCCTTTT
1551	GCAGCCTTTT	TTTTTGGAGA	TTTTCAACGT	GAAAAAATTA	TTATTOGCAA
1601	TTCCTTTAGT	TGTTCCTTTC	TATTCTCACT	COCCTGAAAC	TGTTGAAAGT
1651	TGTTTAGCAA	AACCCCATAC	AGAAAATTCA	TTTACTAACG	TCTGGAAAGA
1701	CGACAAAACT	TTAGATOGTT	ACGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGOGT	TGTAGTTTGT	ACTEGTGACG	AAACTCAGTG	TTACGGTACA
1801	TGGGTTCCTA	ттесесттес	TATOCCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	eegieeeggi	TCTGAGGGTG	GCGGTTCTGA	cecilecoecii	ACTAAACCTC
1901	CTGAGTACGG	TGATACACCT	ATTOOGGGCT	ATACTTATAT	CAACCCTCTC
1951	GACCECACTT	ATOCGOCTEG	TACTGAGCAA	AACCCGCTA	ATOCTAATOC
2001	TICTCTTGAG	GAGTICTICAGC	CTCTTAATAC	TITCATGTTT	CAGAATAATA
2051	GGTTCCGAAA	TAGGCAGGGG	GCATTAACTG	TITATACGGC	CACTGTTACT
2101	CAAGGCACTG	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TATGACGCTT	ACTEGAACEG	TAAATTCAGA	GACTGOGCTT
220		ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
215		TGOCTCAACC	TOCTGTCAAT	GC1GGGGGG	eciciesies
220		CTTTAATCAA	GATOCATTOG	TTTGTGAATA	TCAAGGCCAA
225		TECCTCAACC	TOCTGTCAAT	ecteeceece	ecticitestes
230			AGGGTGGTGG	CICTGAGGGT	GEOGETTUTE
235			GEÓGELLICOS	GIEGIEGCIC	тесттоосст
240		ATGAAAAGAT	GGCAAACGCT	AATAAGGGGG	CTATGACCGA
245			TACAGTOTG	COCTAMAGOC	AAACTTGATT

Figure 5

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2501	CTGTCGCTAC	TGATTACGGT	GCTGCTATCG	ATOGTTTCAT	TGGTGAOGIT
2551	TOOGGOCTTG	CTAATGGTAA	TOGTOCTACT	GGTGATTTTG	CTGGCTCTAA
2601	TTCCCAAATG	GCTCAAGTOG	GTGACGETGA	TAATTCACCT	TTAATGAATA
2651	ATTTOOGTCA	ATATTTACCT	TOOCTOOCTC	AATOGGTTGA	ATGTOGCCCT
2701	TTTGTCTTTA	GOGCTGGTAA	ACCATATGAA	TTTTCTATTG	ATTGTGACAA
2751	AATAAACTTA ,	TTCCGTCGTG	TCTTTGCGTT	TCTTTTATAT	GTTGCCACCT
2801	TTATGTATGT	ATTTTCTACG	TTTGCTAACA	TACTGCGTAA	TAAGGAGTCT
2851	TTATCATGCC	AGTTCTTTTG	GGTATTCCGT	TATTATTGCG	THOCIOGGT
2901	TTCCTTCTGG	TAACTITGIT	COOCTATCTG	CTTACTTTTC	TTAAAAAGGG
2951	CTTCGGTAAG	ATAGCTATTG	CTATTTCATT	GTTICTTGCT	CTTATTATTG
3001	GCCTTAACTC	AATTCTTGTG	GGTTATCTCT	CTGATATTAG	OCCTCAATTA
3051	COCTCTGACT	TIGHTCAGGG	TGTTCAGTTA	ATTICTCCCCGT	CTAATGOGCT
3101	TCCCTGTTTT	TATGTTATTC	TCTCTGTAAA	GCTCCTATT	TTCATTTTTG
3151	ACGTTAAACA	AAAAATCGTT	TCTTATTTGG	ATTGGGATAA	ATAATATGGC
3201	TGTTTATTTT	GTAACTGGCA	AATTAGGCTC	TEGANAGACG	CTOGTTAGOG
3251	TTGGTAAGAT	TCAGGATAAA	ATTGTAGCTG	OCTOCAMANT	AGCAACTAAT
3301	CTTGATTTAA	GCCTTCAAAA	CCTCCCCCAA	GTCGGGAGGT	TOGCTAAAAC
3351 ⁻	COCTOCOCTT	CTTAGAATAC	COGGATAAGCC	TTCTATATCT	GATTTGCTTG
3401	CTATTGGGGG	COGTAATGAT	TOCTACGAATG	AAAATAAAAA	озесттестт
3451	GITCTCGATG	AGTGCGGTAC	TTGGTTTAAT	ACCOGNICIT	GGAATGATAA
3501	CCAAAGACAG	COGATTATTG	ATTGGTTTCT	ACTOCTOGT	AAATTAGGAT
3551	GGGATATTAT	ппспеп	CAGGACTTAT	CTATTGTTGA	TANACAGGCG
3601	COTTICTGCAT	TAGCTGAACA	TGTTGTTTAT	TETOGTOCTO	TGGACAGAAT
3651	TACTITACCT	TTTGTCGGTA	CTTTATATTC	TCTTATTACT	GOCTOGAAAA
3701	теостстеос	TAAATTACAT	eileccelle	TTAAATATGG	OGATTICTCAA
3751	TTAAGCCCTA	CTGTTGAGCG	TTGGCTTTAT	ACTEGTAAGA	ATTTGTATAA
3801	OGCATATGAT	ACTAAACAGG	CTTTTTCTAG	TAATTATGAT	TOOOGTGTTT

Figure 5

3851	ATTCTTATTT	AACGCCTTAT	TTATCACAOG	GTOGGTATTT	CAAAOCATTA
3901	AATTTAGGTC	AGAAGATGAA	ATTAACTAAA	ATAATATTGA	AAAAGTTTTC
3951	TOSOGTTCTT	TGTCTTGCGA	TTGGATTTGC	ATCAGCATTT	ACATATAGTT
4001	ATATAACCCA	ACCTAAGCCG	GAGGITAAAA	AGGTAGTCTC	TCAGACCTAT
4051	GATTTTGATA	AATTCACTAT	TGACTCTTCT	CAGOGTOTTA	ATCTAAGCTA
4101	TOGOTATGTT	TTCAAGGATT	CTAAGGGAAA	TAATTAATTA	AGOGACGATT
4 1'5 1	TACAGAAGCA	AGGTTATTCA	CTCACATATA	TTGATTTATG	TACTGTTTCC
4201	ATTAAAAAAG	GTAATTCAAA	TGAAATTGTT	AAATGTAATT	AATTTIGITT
4251	TCTTGATGTT	TGTTTCATCA	тсптспппа	CTCAGGTAAT	TGAAATGAAT
4301	AATTOGOCTC	TGCGCGATTT	TGTAACTTGG	TATTCAAAGC	AATCAGGGGA
4351	AATOCGTTATT	GITTCICCCG	ATGTAAAAGG	TACTGTTACT	GTATATTCAT
4401	CTGAOGTTAA	ACCTGAAAAT	CTACGCAATT	TOTTTATTTC	TGTTTTACGT
4451	GCTAATAATT	TTGATAATGGT	TEGITCAATT	CCTTCCATAA	TTCAGAAGTA
4501	TAATOCAAAC	AATCAGGATT	ATATTGATGA	ATTGCCATCA	TCTGATAATC
4551	AGGAATATGA	TGATAATTCC	ecicciicig	GIGGITICIT	TGTTCCGCAA
4601	AATĢATAATG	TTACTCAAAC	TTTAAAATT	AATAACGTTC	GGGCAAAGGA
4651	TTTAATACGA	GTTGTCGAAT	TGTTTGTAAA	GTCTAATACT	TCTAAATOCT
4701	CAAATGTATT	ATCTATTGAC	GECTICTAATIC	TATTAGTTGT	TAGTGCTCCT
4751	AAAGATATTT	TAGATAACCT	TOCTCAATTC	CTTTCTACTG	TTGATTTGCC
4801	AACTGACCAG	ATATTGATTG	AGGGTTTGAT	ATTTGAGGTT	CAGCAAGGTG
4851	ATGCTTTAGA	TTTTTCATTT	ectecteect	CTCAGOGTGG	CACTGTTGCA
4901	GEOGRATIA	ATACTGACCG	OCTICACCTICT	GTTTATCTT	CIECTEGIEG
4951	TICGTICGGT	ATTTTTAATG	GOGATGTTTT	AGGGCTATCA	GTTCCCCCCAT
5001	TAAAGACTAA	TAGOCATTCA	AAAATATTGT	CTGTGCCACG	TATTCTTACG
5051	CTTTCAGGTC	AGAAGGGTTC	TATCTCTGTT	GGCCAGAATG	TOCCTTTTAT
5101	TAAAGACTAA	TAGOCATTCA	AAAATATTGT	CTGTGCCACG	TATTCTTACG
5151	OGATTGAGOG	TCAAAATGTA	GGTATTTCCA	TGAGOGTTTT	TOCTGTTGCA

-Figure 5

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5201	ATGGCTGGCG.	GTAATATTGT.	TCTGGATATT	ACCAGCAAGG	COGATAGITT
5251	GAGITCTCT	ACTCAGGCAA	GTGATGTTAT	TACTAATCAA	AGAAGTATTG
5,301	CTACAACGGT	TAATTTGCGT	GATGGACAGA	CTCTTTTACT	COGTOCOCTC
5351	ACTGATTATA	AAAACACTTC	TCAAGATTCT	GEOGTACOGT	TOCTGTCTAA
5401	AATOCCTTTA	ATOGGOCTOC	TGTTTAGCTC	COSCTCTGAT	TOCAMOGAGG
5451	AMAGCACGIT	ATACGTCCTC	GTCAMAGCAA	CCATAGTACG	CECCTGTAG
5501	OGGOGCATTA	AGOGOGGGG	GIGIGGIGGI	TACGCCCAGC	GTGACCGCTA
5551	CACTTGCCAG	COCCTAGCG	COOCCICCTT	TOGOTHOTT	∞σπ∞πί
5601	CTOGOCAOGT	TOGOOGGCTT	TOOOGTCAA	GCTCTAAATC	GEGGGCTCCCC
5651	THAGGGTTC	CGATTTAGTG	CTTTACCGCCA	OCTOGACCCC	AAAAAACTTG
5701	ATTTGGGTGA	TEGTTCACGT	AGTGGGCCAT	COCCTGATA	GACGGTTTTT
5751	CCCCTTTGA	COTTOGAGTC	CACGITCITT	AATAGTGGAC	TCTTGTTCCA
5801	AACTGGAACA	ACACTCAACC	CTATCTOGGG	CTATTCTTTT	GATTTATAAG
5851	GGATTTTGCC	GATTTOGGAA	CCACCATCAA	ACAGGATTTT	COCCTÓCTOG
5901	GGCAAACCAG	OCTTOCACOGC	TTGCTGCAAC	TCTCTCAGGG	CCAGGGGGTG
5951	AAGGGCAATC	AGCTGTTGCC	OCTICIOSCIG	GTGAAAAGAA	AAAOCAOOCT
6001	GEOGCOCAAT	ACCICAMACOG	CTCTCCCCCG	COOCITICACC	GATTCATTAA
6051	TECACCTECC	ACCACAGGIT	TOOOGACTEG	AAAGCCGGCA	GTGAGOGCAA
6101	COCAATTAAT	GTGAGTTAGC	TCACTCATTA	GCCACCCCAG	GCTTTACACT
615	TATGCTTCC	GECTOGTATG	TIGIGIGGAA	TIGIGAGOGG	ATAACAATTT
620	1 CACACAGGAA	ACAGCTATGA	CCATGATTAC	GAATTOGAGC	TOGGTACCOG
625	1 GOGATOCTCT	AGAGTOGACC	TECAGECATG	CAAGCTTGGC	ACTEGEOGTC
630	1 GTTTTACAAC	GTOGTGACTG	GGAAAACCCT	. GEOGTTACCC	AACTTAATOG
635	1 CCTTGCAGCA	CAATCCCCTT	TOGOCAGCTG	GOGTAATAGC	GAAGAGGCCC
640	1 GCACOGATOG	COCTTOCCAA	CAGTTGCGCA	COCTGAATGG	OGAATIGGGGC
645	1 TITECCTEGT	TTOOGGCACC	AGAAGOOGTG	COGGAMAGCT	COCTOCACTG
650	1 CGATCTTCCT	GAGGOOGATA	cegrocarcer	COCCTCAAAC	TEGCAGATEC
					•

Figure 5

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6551	ACCITTACGA	TECCECCATC	TACACCAACG	TAACCTATCC	CATTACGGTC
6601	AATOOGOOGT	TIGTTCCCAC	CCACAATOOG	ACGCGTTGTT	ACTOOCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GGCTACAGGA	ACCOCAGACG	CGAATTATTT
6701	TIGATGGCGT	TOCTATTOGT	TAAAAAATGA	GCTGATTTAA	CAAAAATTTA
6751	ACGCGAATTT	TAACAAAATA	TTAACGTTTA	CAATTTAAAT	ATTIGCTTAT
6801	ACAATCTTCC	TGTTTTGGG	COTTTCTGA	TTATCAACCG	GGGTACATAT
6851	GATTGACATG	CTAGTTTTAC	GATTACCGTT	CATCGATTCT	спатпест
6901	CAGACTICTC	AGGICAATIGAC	CTGATAGOCT	TTGTAGATCT	CTCAAAAATA
6951	CCTACCCTCT	COGGCATGAA	TTTATCAGCT	AGAACGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	COCCECTITIC	TCACCCTTTT	GAATCTTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTTATCCTT	COCTTGAAAT	AMAGGCTTCT	CCCCCAAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	COGATTTAGC	TTTATGCTCT	GAGGCTTTAT

Figure 5

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COMPLEMENTARY TO M₁₃

	•		
POSITION	5' * 3'	POSITION	
645	AGCAACACTATCATA	631	$M_{13}/1$
	•		
615	ACGACGATAAAAACC	601	M ₁₃ /2
585	TTTTGCAAAAGAAGT	571	$M_{13}/3$
300	• •		
555	AATAGTAAAATGTTT	541	M ₁₃ /4
555	• •		
525	CAATACTGCGGAATG	511	M ₁₃ /5
525	• •		
495	TGAATCCCCTCAAA	481	M ₁₃ /6
733			
465	AGAAAAOGAGAATGA	451	M ₁₃ /7
403	* *		
435	CAGGTCTTTACCCTG	421	M 13/8
433	*		
405	AGGAAAGOGGATTGC	391	M ₁₃ /9
700	• — • • • • • • • • • • • • • • • • • •		
375	AGGAAGOOOGAAAGA	361	M ₁₃ /10
0.0		•	

COMPLEMENTARY TO SS PHAGE DNA

POSITION	e' • 3'	POSITION	
351	5' 3' ATATTTGAAGTCTTT	366	M ₁₃ /11
371	TCTTTTGATGCAAT	386	M ₁₃ /12
391	CTATAATACTCAGGG	406	M ₁₃ /13
411	TGATTTATGGTCATT	426	· M ₁₃ /14
431	GTTTAAAGCATTTGA	446	M ₁₃ /15
451	TATTTATGACGATTC	466	M ₁₃ /16
471	TATOCAGTCTAAACA	486	M ₁₃ /17
491	CTCTGGCAAAACTTC	506	M ₁₃ /18
511	TOGOTATTTTGGTTT	526	M ₁₃ /19
·531	AAACGAGGGTTATGA	546	M 13/20

Figure 6

Primers for Nucleic Acid Production Derived from M13mp18 Sequence

12/29

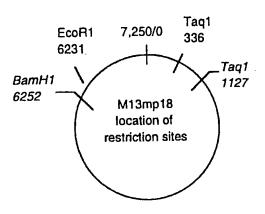


Figure 7

Appropriate M13mp18 Restriction Sites

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Lane 1: from calf thymus + Taq digested mp18 amplification reaction

Lane 2: from Taq digested mp18 amplification reaction

Lane 3: from calf thymus amplification reaction

Lane 4: øX174 Hinf1 size marker

Figure 8

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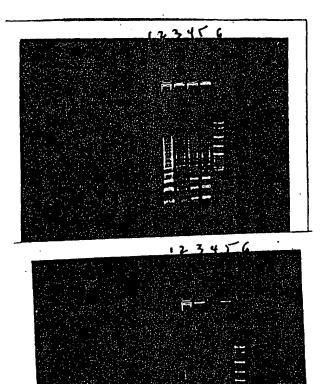
Lane 1: no template

Lane 2: mp18 template, phosphate buffer

Lane 3: Mspl/pBR322 size marker Lane 4: mp18 template, MOPS buffer

Figure 9

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Top= (+) Template
Bottom= (-) Template

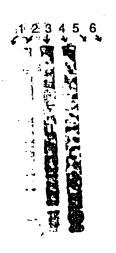
Lane 1: phosphate buffer

Lane 2: MES Lane 3: MOPS Lane 4: DMAB Lane 5: DMG

Lane 6: pBR322/Mspl size marker

Figure 10

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Lane 1: DMAB buffer, no template Lane 2: DMAB buffer, mp18 template Lane 3: DMG buffer, no template

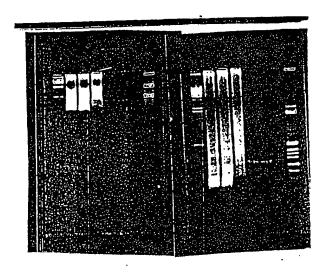
Lane 4: DMG buffer, mp18 template

Lane 5: No reaction

Lane 6: 200 ng Taq I digested mp18 size marker/positive control

Figure 11

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First Time Interval Second Time Interval

Agarose Gel Analysis

Lane 1: lambda Hind III marker

Lane 2: Amp/Untreated

Lane 3: Amp/Kinased

Lane 4: Amp/Kinased/Ligated

Lane 5: PCR/Untreated

Lane 6: PCR/Kinased

Lane 7: PCR/Kinased/Ligated

Lane 8: øX174/Hinf1 marker

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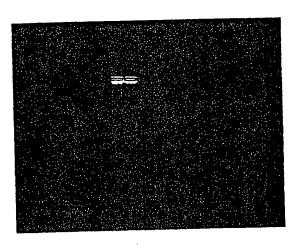
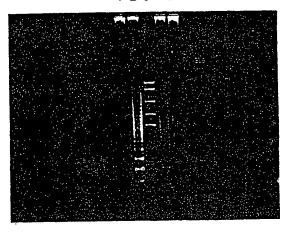


Figure 13

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1 2 3 4 5 6



Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

Lane 4: Primers + RNA

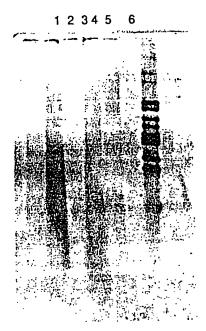
Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Figure 14

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Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

Lane 4: Primers + RNA

. Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Flgure 15



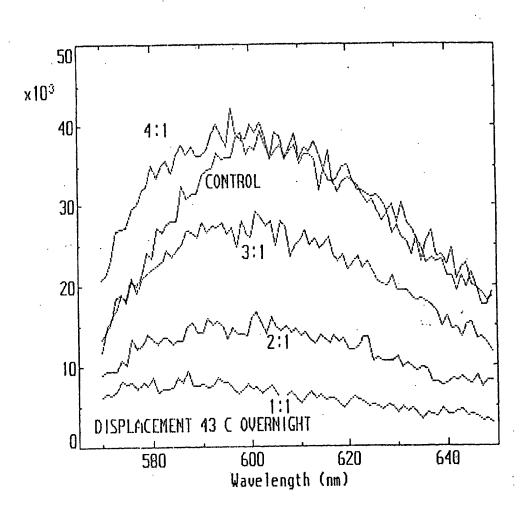


Figure 16

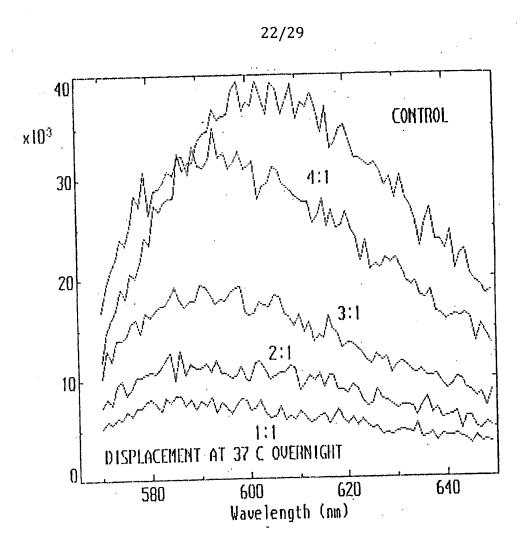


Figure 17

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pIBI 31-BH5-2

fmet AUG of Lac z (T7 Promotor region.... LAC PROMOTOR.ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA

oligo 50-mer 3'- tac t*aa t*gc ggl* ct*a t*ag t*Vt aat* tat* gct* gag t*ga t*at* c-5' 10 base insert

T7 RNA Start («« T3 Promotor Region)
IGGG CTC ICCT TTA GTG ACG GTT AAT
...»») «- T3 Start Signal

pIBI 31 BSII/HCV

(«- T7 Promotor Region)

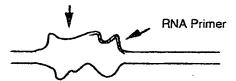
MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....

«- T7 Start Signal
5'-ct'a t'ag t'ga gt'c gt'a tt'a at'..........

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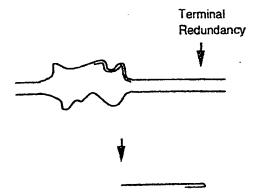
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Replication Bubble with Nucleotide Analogs



Primer-Dependent DNA Production Using Nucleic Acid Construct

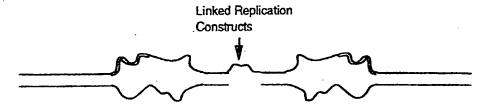
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Hairpin Product

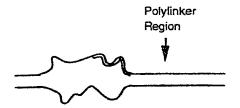
Figure 21

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Linked Complementary Production Constructs

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Cloning Site in Production Constructs

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ARRANGEMENT OF OLIGONUCLEOTIDE PRIMERS IN AMPLIFICATION REACTION

1	2	3	4	5	6	7	8	9	10
20	19	18	17	16	15	14	13	12	11